Polarized Secretion of PEDF from Human Embryonic Stem Cell-Derived RPE Promotes Retinal Progenitor Cell Survival.

Journal: Invest Ophthalmol Vis Sci

Publication Year: 2010

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PubMed link: 21087957

Funding Grants: Therapeutic potential of Retinal Pigment Epithelial cell lines derived from hES cells for retinal degeneration., Stem cell based treatment strategy for Age-related Macular Degeneration (AMD)

Public Summary:
Age-Related Macular Degeneration (AMD) is the most common cause of vision loss in the elderly. AMD is a degenerative disease of the retina, the light sensitive layer of cells at the back of the eye. The retinal pigment epithelium (RPE) is a layer of cells at the outer aspect of the retina that provides support for the light sensitive photoreceptors (PR) and is the initial site of pathology in AMD. In the most common form of AMD, the RPE cells degenerative and die (atrophic AMD or Geographic atrophy). There is currently no effective therapy for late atrophic AMD. Human embryonic stem cell-derived RPE (hESc-RPE) transplantation is a promising therapy for atrophic AMD. We hypothesized that the transplantation of a polarized monolayer of RPE cells that morphologically and functionally mimic native RPE, rather that injection of dissociated RPE cells, would be very important for a successful cell replacement therapy. Thus, we developed a new two-step induction procedure to induce the differentiation of human embryonic cells (hESc) into RPE cells. With this new induction method, we found that pigmented RPE-like cells appeared as early as 4 weeks in culture and were sub-cultured at 8 weeks. These hESc-RPE cells could be expanded in high yield and purity, and continuously developed into functionally polarized hESc-RPE monolayer that exhibit characteristics similar to those of native RPE. However, during the late stages of AMD, there is secondary degeneration and loss of light sensitive PR that would not be replaced with this treatment. Future therapeutic approaches may incorporate the co-transplantation of hESc-RPE with retinal progenitor cells (RPC) as a replacement source for lost photoreceptors as well. We found that polarized hESc-RPE expressed very high levels of pigment epithelium-derived factor (PEDF), a critical agent for the health of normal retinas with its neuroprotective, anti-angiogenic, and anti-senescent functions; and marked increase in the secretion of PEDF into the culture medium as compared to the non-polarized hESc-RPE. When cultured in the presence of supernatants from polarized hESc-RPE, RPCs showed enhanced survival and growth, which was ablated in the presence of anti-PEDF antibody. Our findings demonstrate that hESc can be differentiated into functionally polarized RPE cells that exhibit characteristics similar to those of native RPE. The polarized hESc-RPE also secretes high levels of PEDF that helps to restore the function of remaining photoreceptors and supports RPC survival, which provides strong evidence supporting the co-transplantation of hESc-RPE with photoreceptors or RPCs in the future cell replacement therapy for the late stage of atrophic AMD.

Scientific Abstract:
Purpose: Human embryonic stem cell-derived RPE (hES-RPE) transplantation is a promising therapy for atrophic age-related macular degeneration (AMD); however, future therapeutic approaches may consider co-transplantation of hES-RPE with retinal progenitor cells (RPC) as a replacement source for lost photoreceptors. The purpose of this study was to determine the effect of polarization of hES-RPE monolayers on their ability to promote survival of RPC. Methods: The hES-3 cell line was used for derivation of RPE. Polarization of hES-RPE was achieved by prolonged growth on Transwell inserts. RPC were isolated from 16-18 week gestation human fetal eyes. ELISA was performed to measure pigment epithelial derived factor (PEDF) levels from conditioned media. Results: Pigmented RPE-like cells appeared as early as 4 weeks in culture and were subcultured at 8 weeks. Differentiated hES-RPE had a normal chromosomal karyotype. Phenotypically polarized hES-RPE showed expression of RPE-specific genes. Polarized hES-RPE showed prominent expression of PEDF in apical cytoplasm and a marked increase in secretion of PEDF into the medium compared to non-polarized culture. RPC grown in presence of supernatants from polarized hES-RPE showed enhanced survival, which was ablated in the presence of anti-PEDF antibody. Conclusions: hES-3 cells can be differentiated into functionally polarized hES-RPE cells that exhibit characteristics similar to those of native RPE. Upon polarization, hES-RPE secrete high levels of PEDF that can support RPC survival. These experiments suggest that polarization of hES-RPE would be an important feature for promotion of RPC survival in future cell therapy for atrophic AMD.

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